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In Vitro Interaction of Aminoglycosides with β-Lactam Penicillins

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The aminoglycosides are used clinically in combination with β-lactam antibiotics. The combined use, however, produces an interaction and inactivation of the antibiotics. A study was designed to investigate the kinetics of the interaction in vitro. Four concentrations of aminoglycosides (5 to 20 μg of gentamicin and tobramycin per ml) and penicillins (100 to 600 μg of carbenicillin and ticarcillin per ml) were incubated in plasma (3 days, 37°C). Samples taken at 12-h intervals were analyzed for both aminoglycosides (radioimmunoassay) and penicillin (high-pressure liquid chromatography). In controls, degradation of all four antibiotics were by first-order reactions. In incubation mixtures of two antibiotics, the rate of loss of the aminoglycosides was greater than that in the controls, whereas the rate of loss of penicillins was not significantly increased. The loss of penicillins in incubation mixtures still appeared to be by first-order reactions. However, semilogarithmic plots of aminoglycoside concentrations were curvilinear, suggesting a second-order reaction. Aminoglycoside concentrations in incubation mixtures were fitted by computer to a model incorporating a second-order interaction between aminoglycosides and penicillins and the first-order loss of penicillin from the mixture. The interaction rate constant averaged 2.2×10^{-4} $(\mu g/ml\ h)^{-1}$ for interaction of both carbenicillin and ticarcillin with gentamicin and 1.6×10^{-4} $(\mu g/ml\ h)^{-1}$ for interaction of the penicillins with tobramycin. The effect of the interaction in vivo was examined by computer simulation using the kinetic parameters determined in vitro.

The aminoglycoside antibiotics are often used in combination with β-lactam antibiotics to provide either a wider spectrum of activity against gram-negative bacilli or a synergistic antimicrobial effect against *Pseudomonas aeruginosa* and various enterobacteria. The combined use, however, can result in an interaction and loss of activity of both antibiotics. Since the early reports of inactivation of gentamicin by carbenicillin (6, 19, 26), various aspects of the interaction have been investigated in vitro (4–12, 15, 16, 18, 21–29, 31, 32) and in vivo (2–5, 12, 14, 20, 25, 27, 30, 32, 33). The mechanism of the interaction is thought to involve nucleophilic opening of the β-lactam ring and reaction with an amino group of the aminoglycoside to form an inactive amide (32). In vitro, the interaction is medium (21, 27, 32), temperature (7, 8, 11, 21, 22, 26–28, 31, 32), concentration (23, 24, 26, 27), and time (24, 26, 32) dependent. The interaction is independent of pH within the pH range of 7.4 to 8.0 (10). The extent of inactivation is also dependent on the aminoglycoside (7–10, 23–25, 27, 30) and β-lactam (4, 5, 8, 9, 21–23, 27) used. For example, gentamicin and tobramycin are inactivated to a greater extent than amikacin or netilmicin. Carbenicillin appears to cause greater inactivation than piperacillin. In vivo, the interaction is generally regarded as clinically significant only in patients with impaired renal function (3, 5, 27, 30, 33).

This study was conducted to investigate the kinetics, i.e., to determine the order of the reaction and the interaction rate constants, of the aminoglycoside-β-lactam interaction. Most previous studies have used too few concentrations or have taken samples at too few times to generate any reasonably accurate kinetic data. Although it is well recognized that the concentration of β-lactam antibiotic affects the interaction, very few studies have measured or acknowledged the change in β-lactam concentration over the experimental period. For this study two of the most frequently used aminoglycosides (gentamicin and tobramycin) and β-lactam penicillins (ticarcillin and carbenicillin) were selected. Moreover, concentrations of both antibiotics in incubation mixtures were to be monitored throughout the experiment.

MATERIALS AND METHODS

In vitro study. Stock solutions of gentamicin sulfate, tobramycin, carbenicillin disodium, and ticarcillin disodium (Sigma Chemical Co., St. Louis, Mo.) were mixed with human plasma to yield final concentrations of 5, 10, 15, and 20 μg of gentamicin or tobramycin per ml and 100, 200, 400, and 600 μg of carbenicillin or ticarcillin per ml. These antibiotic mixtures, as well as control samples (i.e., the same concentrations of aminoglycoside or penicillin as a single component in plasma), were incubated at 37°C. Duplicate samples were taken at 12-h intervals for 3 days and frozen immediately (−20°C) until the day of analysis. Duplicate incubation mixtures were prepared for each combination of antibiotic concentrations.

Assay methods. The concentrations of aminoglycoside and penicillin were determined in each plasma sample. Attempts to develop a single method which would accurately analyze

TABLE 1. Degradation rate constants for penicillins

Penicillin	k_P ($h^{-1} \times 10^{-2}$)[a]		
	Control (n[b] = 16)	With gentamicin (n = 32)	With tobramycin (n = 32)
Carbenicillin	1.77 ± 0.41	2.34 ± 0.49	2.15 ± 0.57
Ticarcillin	2.63 ± 0.93	2.71 ± 0.63	3.29 ± 1.1

[a] Values represent the mean ± standard deviation calculated from all concentrations of the penicillin.
[b] n, Number of experiments.

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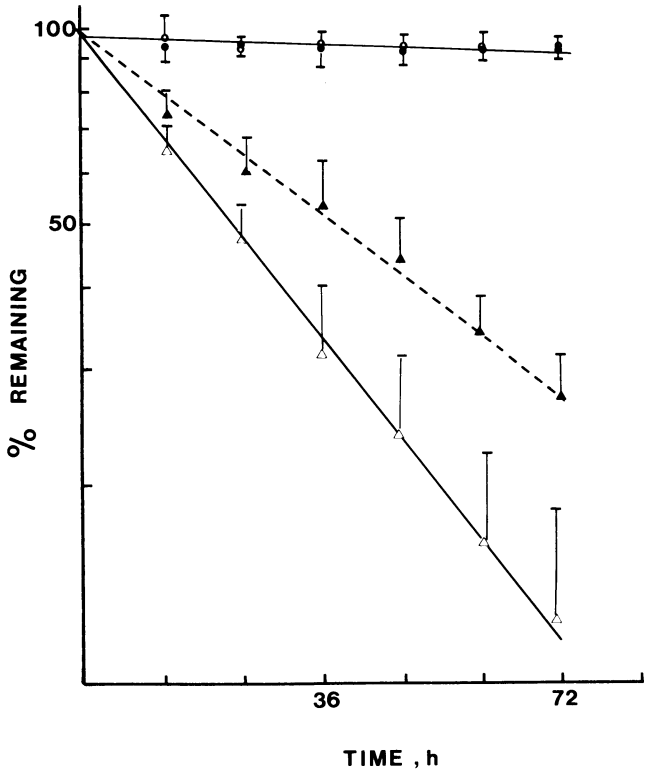

FIG. 1. Semilogarithmic plot of antibiotic degradation in control incubations. Symbols: ●, gentamicin (10 μg/ml); ○, tobramycin (10 μg/ml); ▲, carbenicillin (200 μg/ml); △, ticarcillin (200 μg/ml). Values represent the mean of four incubations. Error bars indicate the SD of the sample.

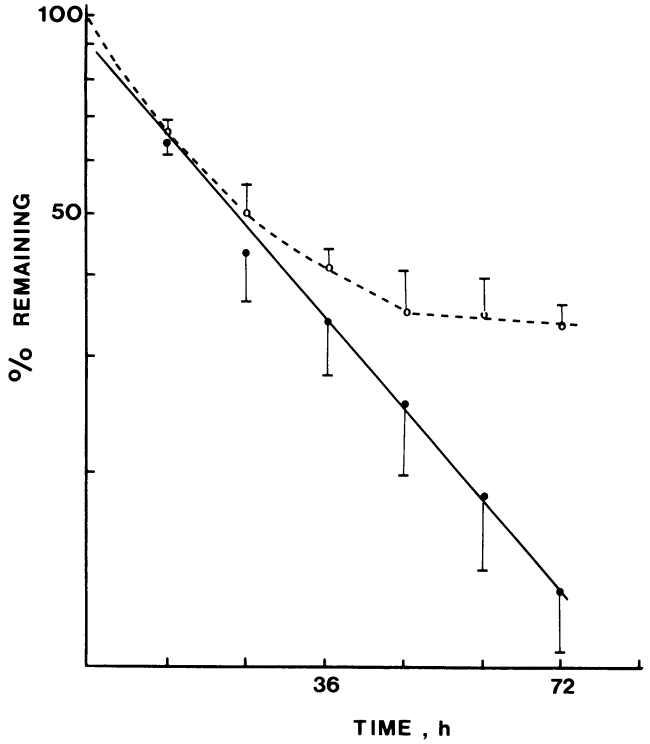

FIG. 2. Semilogarithmic plot of gentamicin (○) and ticarcillin (●) degradation in a mixture containing 10 μg of gentamicin per ml and 200 μg of ticarcillin per ml. Values are averages of duplicate incubations. Error bars indicate the SD of the sample.

both the aminoglycoside and penicillin concentrations in a sample were unsuccessful. Thus, different analytical methods were used for the two groups of antibiotics. Gentamicin and tobramycin concentrations were measured by radioimmunoassay (RIA; RIANEN [^{125}I]gentamicin and [^{125}I]tobramycin RIA kits; New England Nuclear Corp., Lachine, Quebec, Canada). The penicillins did not cross-react in the RIA procedure. The specific cross-reactivity of the interaction product was not determined because no attempt was made to chemically isolate the material. Other investigators have reported a good correlation between RIA and microbiological assays of aminoglycosides in penicillin-aminoglycoside assay mixtures (23–25). Both methods measure what is termed biologically active aminoglycosides (23–25). Carbenicillin and ticarcillin concentrations were determined by high-pressure liquid chromatography by the method of Kwan et al. (13) with a C_{18} reverse-phase column (μ-Bondapak C_{18}; Waters Scientific Ltd., Mississauga, Ontario, Canada) and penicillin G as the internal standard. The retention times for carbenicillin, ticarcillin, and penicillin G (the internal standard) were 4.8, 4.5, and 7.8 min, respectively. The aminoglycosides did not produce any chromatographic peak. A peak, thought to be due to the interaction product, appeared in chromatograms of antibiotic mixtures at a retention time of 2.8 min and did not interfere with quantitation of the penicillins. Extraction efficiencies averaged 82.0% (±0.9% standard deviation [SD]) for carbenicillin and 88.3% (±0.8% SD) for ticarcillin. Stan-

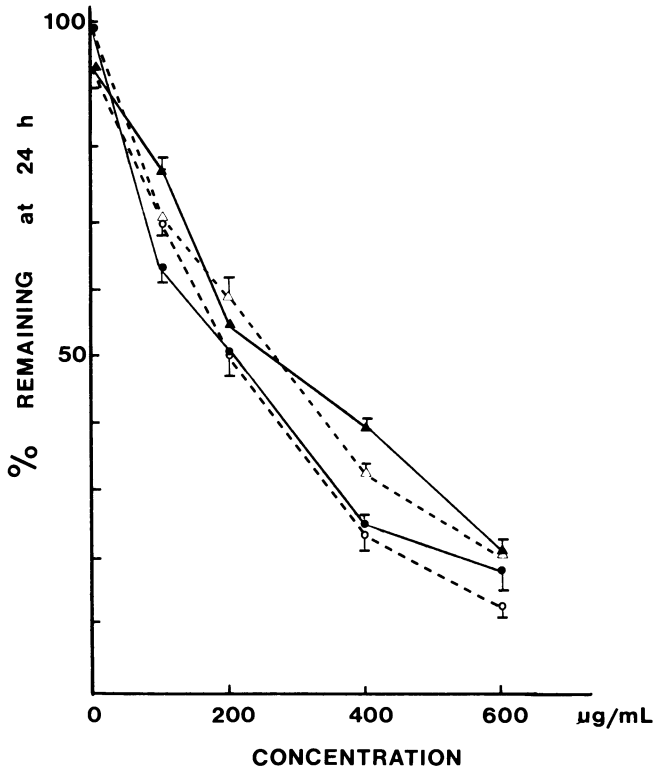

FIG. 3. Effect of penicillin concentration on aminoglycoside degradation, illustrated as the percent aminoglycoside remaining at 24 h for mixtures containing 10 μg of aminoglycoside per ml. Values are averages of duplicate incubations. Error bars indicate the SD of the sample. Symbols: ●, gentamicin-carbenicillin mixture; ○, gentamicin-ticarcillin mixture; ▲, tobramycin-carbenicillin mixture; △, tobramycin-ticarcillin mixture.

dard curves for both RIA and high-pressure liquid chromatographic methods were prepared daily.

Kinetic analysis. From the concentrations of antibiotics in each plasma sample, the percentage of antibiotic remaining at each time was calculated and plotted on both arithmetic and semilogarithmic graph paper. The degradation constants of antibiotics in control samples were calculated from the slope of the decay curve by log-linear regression.

Based on data collected in the study and previously published information, a kinetic model was developed to describe the loss of the drugs in antibiotic mixtures. The model incorporated first-order loss of penicillin and second-order loss of aminoglycoside (i.e., dependent on both the aminoglycoside and penicillin concentrations). Values were obtained for k_p (the degradation rate constants for the penicillins), k_a (the degradation rate constants for the aminoglycosides), and k_i (the interaction rate constant). The k_p value was determined by the log-linear regression of penicillin concentrations with time. Values for k_a and k_i were obtained by computer fitting (MLAB programme; Division of Computer Research and Technology, National Institutes of Health, Bethesda, Md.; DEC-20 computer) of aminoglycoside concentrations (P_0 and k_p previously determined from penicillin data, were specified as constants). Initial estimates of k_i and k_a were obtained by graphical techniques (see Appendix). The time required to lose 50% of the original antibiotic concentration was determined from graphs of the percent remaining versus time.

Statistical analysis. The effects of concentrations and the various antibiotic combinations on degradation were examined by analysis of variance (ANOVA) and Newman-Keuls multiple range tests. Differences were considered statistically significant if $P < 0.05$.

Computer simulations. Plasma concentrations after simultaneous administration of gentamicin and carbenicillin in vivo were simulated by computer using the MLAB programme on a DEC-20 computer. Simulations were performed by integrating the differential equations for aminoglycoside (A) and penicillin (P) concentrations from time zero to 48 h by using 2-h increments. Simulations were based on k_i values determined in the study and literature values for the disposition rate constants of gentamicin [$k_{d(A)}$] and carbenicillin [$k_{d(P)}$]. The disposition rate constants in patients with various degrees of renal dysfunction were estimated by multiplying the normal disposition rate constant (0.35 h^{-1} for gentamicin; 0.59 h^{-1} for carbenicillin) (1, 17) by kidney function (expressed as a fraction of normal).

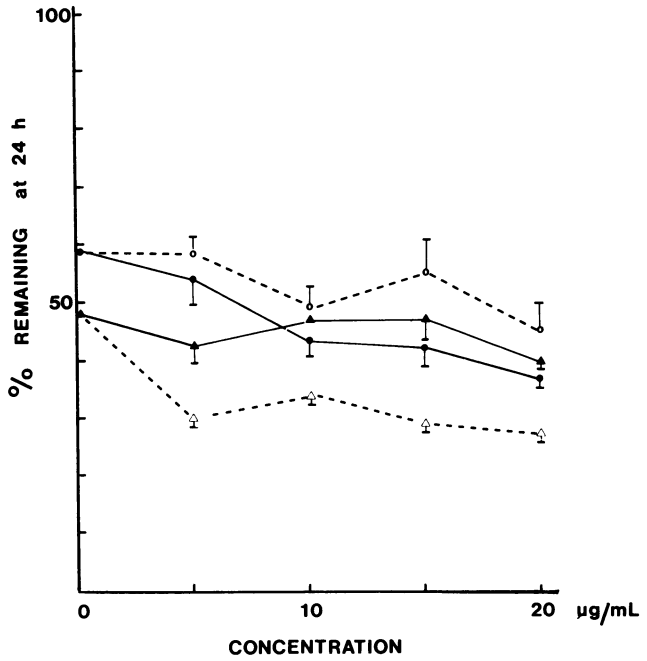

FIG. 4. Effect of aminoglycoside concentration on penicillin degradation, illustrated as the percent penicillin remaining at 24 h for mixtures containing 200 µg of penicillin per ml. Symbols: ●, carbenicillin-gentamicin mixture; ○, carbenicillin-tobramycin mixture; ▲, ticarcillin-gentamicin mixture; △, ticarcillin-tobramycin mixture. Values are averages of duplicate incubations. Error bars indicate the SD of the sample.

TABLE 2. Times required for 50% loss (t_{50}) of aminoglycosides in incubation mixtures

Aminoglycoside-penicillin	Initial penicillin concn (µg/ml)[a]	t_{50} at the following initial aminoglycoside concn (µg/ml): 5	10	15	20	t_{50} (mean ± SD)
Gentamicin	0	44.8 ± 31.3 days	47.0 ± 10.0 days	27.3 ± 12.2 days	29.0 ± 7.0 days	36.3 ± 17.7 days
Gentamicin-carbenicillin	100	>72 h	>72 h	>72 h	>72 h	
	200	24.7 ± 4.3 h	24.3 ± 3.8 h	28.4 ± 5.5 h	36.8 ± 8.3 h	28.5 ± 6.9 h
	400	10.0 ± 1.4 h	8.6 ± 1.0 h	11.0 ± 1.4 h	10.3 ± 1.4 h	10.0 ± 1.4 h
	600	6.5 ± 1.5 h	5.9 ± 0.2 h	7.3 ± 0.4 h	7.1 ± 0.5 h	6.7 ± 0.8 h
Gentamicin-tricarcillin	100	>72 h	>72 h	>72 h	>72 h	
	200	29.0 ± 4.2 h	26.0 ± 6.5 h	24.8 ± 0.6 h	26.0 ± 3.5 h	26.4 ± 3.7 h
	400	8.4 ± 0.8 h	9.7 ± 0.9 h	10.6 ± 1.0 h	9.0 ± 0.9 h	9.4 ± 1.1 h
	600	6.9 ± 0.8 h	4.7 ± 0.6 h	5.1 ± 1.0 h	6.4 ± 0.3 h	5.8 ± 1.2 h
Tobramycin	0	29.0 ± 9.4 days	39.3 ± 37.8 days	31.0 ± 27.7 days	28.9 ± 16.2 days	32.4 ± 23.3 days
Tobramycin-carbenicillin	100	>72 h	>72 h	>72 h	>72 h	
	200	29.5 ± 0.7 h	44.7 ± 20.8 h	40.5 ± 4.3 h	>72 h	
	400	15.6 ± 0.8 h	15.6 ± 0.5 h	12.5 ± 1.4 h	15.1 ± 2.6 h	14.7 ± 1.8 h
	600	10.7 ± 0.5 h	9.1 ± 1.2 h	7.2 ± 0.4[b] h	8.7 ± 0.1 h	8.9 ± 1.4 h
Tobramycin-ticarcillin	100	>72 h	>72 h	>72 h	>72 h	
	200	>72 h	>72 h	50.1 ± 1.3 h	63.8 ± 10.0 h	
	400	21.2 ± 1.8 h	11.8 ± 0.6 h	10.4 ± 1.8 h	9.8 ± 1.2 h	13.3 ± 5.1 h
	600	9.5 ± 0.2[b] h	7.0 ± 0.7 h	6.8 ± 0.3 h	5.4 ± 1.2 h	7.2 ± 1.7 h

[a] t_{50} values at all penicillin concentrations tested were significantly different.
[b] t_{50} value was significantly different from those at other aminoglycoside concentrations ($P < 0.05$ by ANOVA and Newman-Keuls multiple comparison test).

TABLE 3. Interaction rate constants for gentamicin or tobramycin and carbenicillin or ticarcillin

Aminoglycoside-penicillin mixture	Initial penicillin concn	k_i (ml/μg h × 10^{-4}) at the following initial aminoglycoside concn:				k_i (mean ± SD)
		5	10	15	20	
Gentamicin-carbenicillin	100	1.69	2.80	2.65	2.39	2.38 ± 0.47
	200	2.28	2.58	2.53	2.36	2.44 ± 0.36
	400	2.20	1.80	2.36	2.15	2.13 ± 0.39
	600	1.42	1.58	1.75	1.82	1.64 ± 0.23[a]
	(Mean ± SD)	1.90 ± 0.50	2.19 ± 0.58	2.32 ± 0.43	2.18 ± 0.35	2.14 ± 0.48
Gentamicin-ticarcillin	100	1.96	2.41	3.12	3.89	2.84 ± 0.99
	200	2.14	2.53	2.67	2.56	2.47 ± 0.30
	400	1.68	2.22	2.56	2.50	2.24 ± 0.48
	600	1.09	1.92	1.89	1.81	1.68 ± 0.41[a]
	(Mean ± SD)	1.72 ± 0.56[a]	2.27 ± 0.32	2.56 ± 0.64	2.69 ± 0.91	2.31 ± 0.72
Tobramycin-carbenicillin	100	2.27	1.67	1.89	2.41	2.06 ± 0.51[a]
	200	1.24	1.48	1.40	1.40	1.38 ± 0.35
	400	1.25	1.20	1.25	1.61	1.32 ± 0.30
	600	1.30	1.29	1.48	1.67	1.43 ± 0.18
	(Mean ± SD)	1.51 ± 0.59	1.41 ± 0.31	1.50 ± 0.32	1.75 ± 0.53	1.55 ± 0.45
Tobramycin-ticarcillin	100	1.40	2.24	1.32	2.19	1.79 ± 1.57
	200	1.27	1.53	1.68	1.96	1.61 ± 0.35
	400	0.90	1.67	1.73	1.98	1.57 ± 0.44
	600	1.14	1.55	1.66	1.96	1.58 ± 0.32
	(Mean ± SD)	1.18 ± 0.50[a]	1.75 ± 0.74	1.60 ± 0.55	2.02 ± 1.19[a]	1.64 ± 0.81

[a] k_i value significantly different from values at other aminoglycoside concentrations or at other penicillin concentrations ($P < 0.05$ by ANOVA and Newman-Keuls multiple comparison test).

RESULTS

In control incubations of a single antibiotic in plasma at 37°C, carbenicillin and ticarcillin decayed rapidly. Approximately 50 to 60% of the original concentration remained after 24 h.Degradation of the penicillins appeared to be by a first-order reaction (Fig. 1), with an average half-life of 39 h for carbenicillin and 26 h for ticarcillin over the concentration range of 100 to 600 μg/ml. Degradation was more rapid, and the rate constant was larger for ticarcillin than for carbenicillin (Table 1). Compared with the penicillins, the aminoglycoside antibiotics were relatively stable when incubated alone in plasma (Fig. 1). The loss at 24 h was less than 10%. Degradation half-lives were estimated as 30 to 50 days, but accuracy of the values was limited by the minor loss of drug over the 3-day period. Under the conditions used in the study there did not appear to be any major differences in degradation of tobramycin and gentamicin in control samples (Fig. 1).

In antibiotic mixtures, the degradation of carbenicillin or ticarcillin remained a first-order reaction (Fig. 2). Degradation rate constants were only slightly larger than in controls (Table 1). The degradation of gentamicin and tobramycin, however, increased significantly when incubated with one of the penicillins (Fig. 2). Loss of aminoglycoside at 24 h ranged from 20 to 80% (in contrast to less than 10% in controls) and was dependent on the concentration of penicillin (Fig. 3). For example, at penicillin concentrations of 100 μg/ml, more than 50% of the original gentamicin concentration remained at the end of the 3-day incubation period. A 50% loss occurred in 20 to 30 h with 200 μg of the penicillins per ml and in 6 to 10 h with 400 to 600 μg/ml. The initial aminoglycoside concentration had little effect on the degradation of the penicillins (Fig. 4) or the inactivation of the aminoglycoside itself (Table 2).

Degradation curves of aminoglycosides in antibiotic mixtures were obviously nonlinear both on arithmetic and semilogarithmic graph paper (Fig. 2). The slope of the terminal portion of the curve was significantly less than the initial slope and appeared to parallel the degradation of aminoglycosides in control samples.

Computer fitting of aminoglycoside concentrations in antibiotic mixtures to the model described (Appendix) yielded

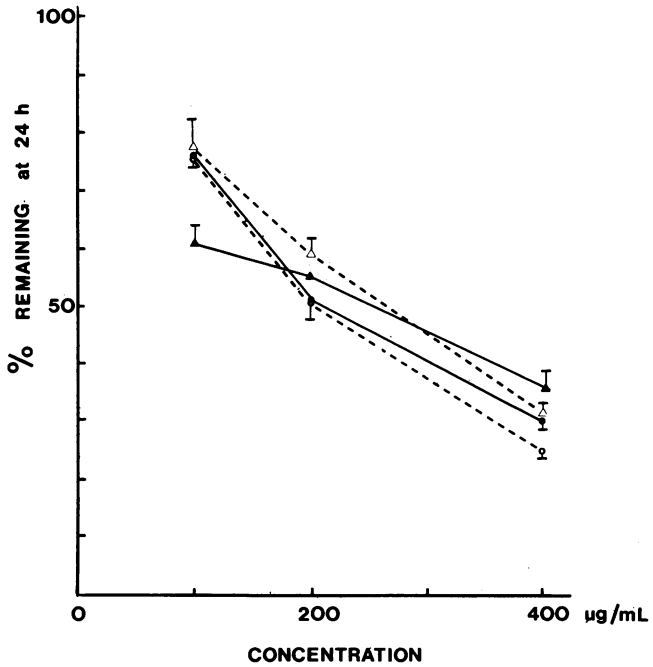

FIG. 5. Effect of penicillin concentration on aminoglycoside degradation for mixtures containing the same ratio (20:1) of initial antibiotic concentrations. Each mixture initially contained 5, 10, or 20 μg of the aminoglycoside per ml with 100, 200, or 400 μg of the penicillin per ml. Symbols: ●, gentamicin-carbenicillin mixture; ○, gentamicin-ticarcillin mixture; ▲, tobramycin-carbenicillin mixture; △, tobramycin-ticarcillin mixture. Values are averages of duplicate determinations. Error bars indicate the SD of the sample.

TABLE 4. Interaction rate constants as reported previously

Reference	Experimental conditions	Antibiotic	Interaction rate constants[a]	
			K_i (h^{-1})	k_i (h^{-1} μg^{-1} ml)
O'Bey et al. (22)	In vitro, serum, 37°C	Tobramycin (8 μg/mL); carbenicillin (200 μg/mL)	0.0088 ± 0.0013	0.4×10^{-4}[b]
Ervin et al. (5)	In vivo, 17 patients with end-stage renal disease	Gentamicin (1 mg/kg) with ticarcillin (40 mg/day)	0.01	0.6×10^{-4}[b]
	Intravenous infusion of gentamicin over 0.5 h with penicillin following	Gentamicin (1.5 mg/kg with carbenicillin (75 mg/day)	0.016 ± 0.004	0.5×10^{-4}[b]
Konishi et al. (12)	In vivo, 6 healthy adults	Tobramycin (80 mg) with carbenicillin (5 g)	0.047 ± 0.039	1×10^{-4}[b]
	Intravenous infusion of tobramycin over 1 h, penicillin added to aminoglycoside immediately before infusion	Tobramycin (80 mg) with ticarcillin 5 g	0.042 ± 0.026	1×10^{-4}[b]
Thompson et al. (30)	In vivo, 12 patients with chronic renal failure Intravenous infusion of gentamicin over 0.5 h with penicillin following	Gentamicin (2 mg/kg) carbenicillin (2 g) every 8 h	0.025 ± 0.011	1×10^{-4}[b]
Present study	In vitro, plasma, 37°C	Gentamicin 5–20 μg/ml with carbenicillin or ticarcillin (100–600 μg/ml)	0.022–0.132[c]	2.2×10^{-4}
		Tobramycin 5–20 μg/ml with carbenicillin or ticarcillin (100–600 μg/ml)	0.016–0.096[c]	1.6×10^{-4}

[a] K_i, First-order rate constant; k_i, second-order rate constant.
[b] Calculated from reported K_i value and initial penicillin concentration, P_0; $k_i = K_i\ (h^{-1})\ P_0\ (\mu g/ml)$.
[c] Calculated from k_i and initial penicillin concentration, P_0; $K_i = k_i\ (h^{-1}\ \mu g^{-1}\ ml)\ P_0\ (\mu g/ml)$.

an average second-order interaction rate constant (k_i) of 2.2×10^{-4} μg^{-1} h^{-1} ml for gentamicin and the penicillins and 1.6×10^{-4} μg^{-1} h^{-1} ml for tobramycin. Although loss of the aminoglycosides was dependent on the concentration of carbenicillin or ticarcillin (Table 2), k_i was largely independent of the concentrations of both aminoglycosides and penicillins (Table 3). Overall, the k_i values for the interaction of gentamicin or tobramycin with carbenicillin were not significantly different (ANOVA, $P > 0.05$) from those with ticarcillin. However, the k_i for gentamicin was larger than that of tobramycin.

DISCUSSION

As previously reported by other investigators, loss of aminoglycosides over the 3-day incubation period was significantly greater in the presence of penicillin antibiotics. Results of early studies by Riff and Jackson (26, 27) have suggested that the rate of inactivation was influenced by the relative concentration of the two antibiotics. In the present study, with four different concentrations of all antibiotics, more than one combination generated the same ratio of penicillin to aminoglycoside concentrations; e.g. 100 μg of penicillin per ml with 5 μg of aminoglycoside per ml, 200 μg of penicillin per ml with 10 μg of aminoglycoside per ml, and 400 μg of penicillin per ml with 20 μg of aminoglycoside per ml, all yielded a concentration ratio of 20:1. The rate of inactivation for the same ratio of initial concentrations was higher with higher penicillin concentrations (Fig. 5). Data reported by Pickering and Rutherford (Table 1 in reference 24) showed similar results.

The rate of loss of aminoglycosides in antibiotic mixtures could not be described by a first-order reaction: graphs of the percent remaining with time were nonlinear even when plotted on semilogarithmic graph paper. This nonlinearity only becomes noticeable if concentrations are measured frequently. Although not discussed, there is evidence of such nonlinearity in earlier data presented in the literature (5, 12, 22). O'Bey et al. (22) have fitted tobramycin concentrations determined over a 48-h period by log-linear regression. There are, however, systematic deviations of the data from the fitted line, indicating that the data should perhaps be fitted to some function other than monoexponential decay.

The decay curves in vitro could be described mathematically by a series of exponentials, e.g., $Ae^{-\alpha t} + Be^{-\beta t}$. This approach, however, would not isolate or characterize an interaction constant which could be used to predict inactivation rates at different antibiotic concentrations or in different clinical situations. The chemistry of the interaction (30, 32), its dependence on penicillin concentration, and the nonlinearity of the degradation profile suggest that the interaction could be described as a second-order reaction. A model combining first-order loss owing to the interaction also generated a biphasic decay curve and seemed to have wider applicability for clinical situations. This was the model used to fit data obtained in our in vitro experiments.

To fit aminoglycoside concentrations to such a model, penicillin concentrations must also be measured. Although investigators have consistently documented a correlation between the extent of the interaction and penicillin concentration, the observations have been based on the initial penicillin concentration. Few studies have measured both aminoglycoside and penicillin concentrations throughout the incubation period. Ervin et al. (5) did measure levels of

TABLE 5. Computer simulation[a] of carbenicillin and gentamicin concentrations after simultaneous administration to patients with various degrees of renal function[b]

Renal function	Initial carbenicillin concn	Time after administration (h)[c]	Gentamicin concn (μg/ml)		Carbenicillin concn (μg/ml)	
			Without interaction	With interaction	Without interaction	With interaction
Normal renal function	100	0	5.00	5.00	100	100
$t_{1/2}$ (gentamicin) 2 h		4	1.23	1.20	6.3	6.3
$t_{1/2}$ (Carbenicillin)1 h		8	0.30	0.30	0.4	0.4
	400	0	5.00	5.00	400	400
		4	1.23	1.11	25	25
		8	0.30	0.27	1.6	1.6
Impaired renal function						
20% renal function	100	0	5.00	5.00	100	100
$t_{1/2}$ (gentamicin) 10 h		12	2.18	1.96	19	19
$t_{1/2}$ (carbenicillin) 5 h		24	0.95	0.84	3.5	3.4
	400	0	5.00	5.00	400	400
		12	2.18	1.41	75	74
		24	0.95	0.57	14	14
10% Renal function	100	0	5.00	5.00	100	100
$t_{1/2}$ (gentamicin) 20 h		12	3.29	2.82	44	43
$t_{1/2}$ (carbenicillin) 10 h		24	2.16	1.73	19	19
		48	0.93	0.72	3.6	3.6
	400	0	5.00	5.00	400	400
		12	3.29	1.77	175	174
		24	2.16	0.89	76	76
		48	0.93	0.32	15	14
5% Renal function	100	0	5.00	5.00	100	100
$t_{1/2}$ (gentamicin) 40 h		12	4.03	3.35	66	65
$t_{1/2}$(carbenicillin) 20 h		24	3.25	2.39	43	42
		48	2.11	1.36	19	18
	400	0	5.00	5.00	400	400
		12	4.03	1.92	263	261
		24	3.25	0.95	173	171
		48	2.11	0.36	75	74

[a] Computer simulation (MLAB programme package) for differential equations: $dA/dt = -k_{d\,(A)}A - k_iA, P$, $dP/dt = -k_{d\,(P)}P - k_iA \cdot P$ with a value of 1.9×10^{-4} ml/μg h for k_i (mean value for a gentamicin concentration of 5 μg/ml [Table 3]).

[b] A range of renal function from 100 to 5% of normal; $k_{d\,(P)}$ and $k_{d\,(A)}$ values calculated based on an average half-life ($t_{1/2}$) for gentamicin of 2 h and for carbenicillin of 1 h in patients with normal renal function.

[c] A 2-h interval was used for computer simulation, but data are reported for only selected intervals, corresponding to dosing intervals which might be used for various degrees of renal function.

gentamicin, ticarcillin, and carbenicillin, and they noted a slowing of gentamicin inactivation when penicillin concentrations fell below 50 to 100 μg/ml. Some investigators have compensated for the degradation of the penicillins by the intermittent addition of penicillins to incubation mixtures (5, 28).

The model also explains the lack of effect of aminoglycoside concentration on the loss of penicillins observed experimentally. Since the concentration of the penicillins far exceeded that of the aminoglycosides, degradation of penicillin [i.e., the term k_pP represented a more significant source of loss than the interaction [i.e., $k_iA \cdot P$]. This should also be the case in clinical situations because penicillins are used in much larger doses and achieve much higher concentrations in plasma than do aminoglycosides.

In some studies in which the interaction in vivo was investigated, an interaction rate constant has been calculated. In all cases, this constant has been calculated as the difference in the elimination rate constant when the aminoglycoside was given alone (β) and when given with a penicillin (β_p), i.e., $K_i = \beta_p - \beta$. This approach, however, implies that the interaction constant, like the elimination rate constant, describes a first-order reaction, with the rate of reaction being proportional to the aminoglycoside concentration. Such a calculation ignores the myriad of findings that the interaction rate is also dependent on the penicillin concentration. It could be argued that a K_i calculated in this manner represents a pseudo-first-order rate constant, i.e. $K_i = k_iP_0$, where P_0 represents the initial penicillin concentration. For this argument to be valid, penicillin concentrations would have to remain relatively constant. This major difference between results of the present and previous studies, in the analysis of data and calculation of an interaction rate constant, makes comparisons of k_i values difficult. To allow for some comparison, k_i values from this study were used to calculate an initial interaction constant (k_iP_0). This value, based on the initial penicillin concentration, thus would have the same units of measurement ($hours^{-1}$) as interaction rate constants reported by previous investigators. The constants calculated in this manner, ranging from 0.022 to 0.032 h^{-1} for P_0 values of 100 to 600 μg/ml, were in the same order of magnitude as K_i values reported previously (Table 4).

An initial illustration of the clinical implications of this study is provided by computer simulations of plasma concentrations after single, simultaneous intravenous doses of gentamicin and carbenicillin were administered to patients who had various degrees of renal dysfunction (Table 5). These conditions would result in the maximum interaction of antibiotics. Simulations were based on a normal elimination half-life of 1 h for carbenicillin (17) and 2 h for gentamicin (1).

The first-order elimination rate constant [$k_{d(P)}$ or $k_{d(A)}$] replaces the first-order degradation rate constant (k_P or k_A) of the model, and k_i remains to describe the second-order interaction. The effect of the interaction on penicillin concentrations is negligible. With normal renal function, the difference in gentamicin concentration at any time during an 8-h period would be less than 0.1 μg/ml for an initial carbenicillin concentration of 100 μg/ml. As renal function decreases, the decrease in concentration produced by the interaction becomes larger, increasing from ca. 0.2 μg/ml for 20% kidney function to 0.8 μg/ml with 5% kidney function. The larger the initial penicillin concentration the greater the difference becomes (note a difference of over 2 μg/ml at a carbenicillin concentration of 400 μg/ml with a kidney function 5%). Such differences would accumulate on multiple dosing. Simulations of multiple dosing at various dosing intervals are now under way.

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APPENDIX A

Control incubations. The first-order degradation of antibiotics was as follows:
Penicillins:

$$\frac{dP}{dt} = -k_P P \qquad \ln(P/P_0) = -k_P t \tag{1}$$

Aminoglycosides:

$$\frac{dA}{dt} = -k_A A \qquad \ln(A/A_0) = -k_A t \tag{2}$$

where t is incubation time; P and P_0 are the concentrations of penicillin at times t and zero, respectively; A and A_0 are the concentrations of aminoglycoside at times t and zero, respectively; k_P and k_A are first-order degradation constants for penicillin and aminoglycosides, respectively.

Interaction experiments. (i). Loss of antibiotics is a sum of first-order degradation and a second-order interaction, as shown below:
Aminoglycosides:

$$\frac{dA}{dt} = -k_A A - k_i A \cdot P \tag{3}$$

Penicillins:

$$\frac{dP}{dt} = -k_P P - k_i A \cdot P \tag{4}$$

where k_i is the second order interaction rate constant.

(ii). To integrate and solve Eq. 3, assume that $k_P P >> k_i A \cdot P$, then:

$$\frac{dP}{dt} = -k_P P \qquad P = P_0 e^{-k_P t} \tag{5}$$

(A plot of lnP with time yielded a straight line with a slope of $-k_P$ and an intercept of P_0.)

Substitute Eq. 5 into Eq. 3:

$$\frac{dA}{dt} = -k_A A - k_i A \cdot P_0 e^{-k_P t} \tag{6}$$

Integration of Eq. 6 gives:

$$A = A_0 \exp\left(-k_A t + \frac{k_i P_0 e^{-k_P t}}{k_P} - \frac{k_i P_0}{k_P}\right) \tag{7}$$

(A plot of lnA with time yielded a curve with a terminal slope approximately parallel to that of control incubations.)

(iii). For initial estimates of k_A and k_i (for computer fitting), as $t \to \infty$, $e^{-k_P t} \to 0$, therefore, Eq. 7 becomes:

$$A = A_0 \exp\left(-k_A t - \frac{k_i P_0}{k_P}\right) \qquad \ln(A/A_0) = -k_A t - \frac{k_i P_0}{k_P} \tag{8}$$

The terminal portion of the curve, therefore, has a slope of $-k_A$ and an intercept ($t = 0$) of $-k_i P_0/k_P$.

Values for P_0 and k_P were determined previously from measurements of penicillin concentrations with time and Eq. 5. The initial estimate of k_i, therefore, was calculated as $k_i = (-\text{intercept})k_P/P_0$.

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